

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 09/772,445
Applicant : KLEINMAN et al.
Filed : January 29, 2001
TC/A.U. : 1654
Examiner : Ronald T. Niebauer

Docket No. : 2600-109
Customer No. : 06449
Confirmation No. : 1045

DECLARATION UNDER 37 C.F.R § 1.132

Director of the United States Patent
and Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, H. Paul Ehrlich, declare that:

1. I am a professor of Surgery and Cellular and Molecular Physiology at the Hershey Medical Center, Penn State University College of Medicine. I was awarded a Ph.D. degree from the University of California at San Francisco. I am a recognized expert in the field (my *Curriculum Vitae* is attached as Exhibit A). I am on the Editorial Advisory Board of the journals *Wound Repair and Regeneration* and *Journal of Burn Care and Rehabilitation*. In 2010, I received the Lifetime Achievement Award from the Wound Healing Society. I have studied wound healing, tissue repair and tissue regeneration for over three decades. I have also studied the properties and therapeutic actions of thymosin peptides and have conducted and/or supervised many laboratory studies over that period of time. I also have authored or co-authored books and numerous scientific papers in this area. A copy of my curriculum vitae is attached.

2. I have reviewed and am familiar with U.S. Patent Application Serial Number 09/772,445 entitled "Compositions and Methods for Promoting Wound Healing and Tissue Repair."
3. I understand that the claims of the above-referenced U.S. patent application have been rejected on the basis of an article by S.N. Turischev, which has been translated from Russian and the translation has been supplied to the applicants by the U.S. Patent and Trademark Office, entitled "[Study of the effect of thymosin on the healing of flat skin wounds in rats," as evidenced by U.S. Patent No. 6,030,948 to Mann, or in combination with other references.
4. I have been informed that it is the position of the U.S. Patent and Trademark Office in an Office Action dated January 11, 2011, that Turischev describes experiments where Thymosin Fraction 5 (hereinafter "TF5") was administered either intraperitoneally or topically to rats having skin wounds, and that Turischev disclosed that there is acceleration of the healing rates at a dose of 0.8 ug/g TF5 by intraperitoneal administration.
5. I have been informed that the U.S. Patent and Trademark Office has acknowledged that Turischev does not disclose that TF5 contains thymosin beta 4 (hereinafter "T β 4"), but that Mann (U.S. 6,030,948) disclosed that TF5 contains T β 4 and thymosin alpha 1.
6. I have studied the English translation of the Turischev article and the Mann (U.S. 6,030,948) patent. For the following reasons, I believe that:
 - a) Turischev demonstrates that the effect of administering TF5 is unpredictable depending on the mode of administration;

b) Turischev discloses that the use of TF5 topically was detrimental to the wound healing process in rats;

c) Turischev demonstrates that only one out of the three tested dosage amounts of TF5 administered intraperitoneally to the rats, i.e., 0.8 µg/g, had a significant effect on wound healing;

d) Turischev demonstrates that increasing or decreasing the sole effective dosage amount resulted in a loss of effectiveness for accelerating wound healing compared to control;

e) it was unpredictable, in view of Mann, whether the effect demonstrated by Turischev was attributable to Tβ4, to thymosin alpha 1, or to a combination thereof; and

f) Turischev's method is directed specifically to accelerating skin wound healing, but not to a method for promoting tissue repair.

7. It is my expert opinion that persons of ordinary skill in the art reading Turischev as evidenced by Mann, would have found the results confounding and been unable to extract suggestions for obvious modifications and practical methods of using TF5. Persons of ordinary skill in the art would have found that it was unpredictable whether another form or administration of TF5 would have a positive effect (e.g., intraperitoneal at 0.8 µg/g), a negative effect (e.g., topical administration at 0.8 µg/g or 1.6 µg/g), or any effect at all (e.g., intraperitoneal administration at 0.2 µg/g or 1.6 µg/g) on wound healing.

8. It is my expert opinion that persons of ordinary skill in the art reading Turischev as evidenced by Mann, would have been dissuaded from using TF5 topically because Turischev discloses that TF5 administered topically caused “intensification and prolongation of the phase of inflammation” (page 4, lines 15-17), increased suppuration with increased TF5 dosage administered (page 4, lines 13-15), significantly slower healing and slower contraction of wounds compared to control (page 3, last two paragraphs), and “a trend toward lengthening of [the period for complete epithelialization (PCE)]” with increasing TF5 dose administered (page 5, lines 1-3).
9. It is my expert opinion that persons of ordinary skill in the art reading Turischev as evidenced by Mann, would have been dissuaded from using TF5 intraperitoneally at a dose other than 0.8 µg/g. Turischev discloses that dosages of 0.2 µg/g and 1.6 µg/g did not significantly accelerate wound healing compared to control (page 3, lines 5-15). Thus, persons of ordinary skill in the art would have found it useless to administer TF5 at a dose lower or higher than 0.8 µg/g.
10. It is my expert opinion that persons of ordinary skill in the art reading Turischev as evidenced by Mann, would not have known whether the effect on wounds observed at 0.8 µg/g TF5 was due to the activity of Tβ4, the activity of thymosin alpha 1, or the combined activity of Tβ4 and thymosin alpha 1. Mann discloses that “Tα1 and Tβ4 have been characterized with regard to their ability to...enhance wound healing....” Col. 4, lines 55-58. Accordingly, persons of ordinary skill in the art reading Mann would have been unable to determine what effect, if any, was attributable to Tβ4. Thus, persons of ordinary skill in the art would not have had any guidance as to how to use Tβ4 in a composition, e.g., at what concentration, at what dosage, alone or combined

with Tα1 and other thymus ingredients. Accordingly, it is my expert opinion that Turischev cannot be read as providing anything more than instructions to use TF5 at 0.8 µg/g intraperitoneally and no suggestions for modification or optimization of this method. The confounding results of Turischev compel this narrow reading.

11. Persons of ordinary skill in the art reading Turischev as evidenced by Mann, would have found Turischev's method at odds with a method for promoting tissue repair. It is my expert opinion, as evidence by Turischev, that there is a technical difference between the terms "wound healing" and "tissue repair." Turischev discloses that "[t]he use of T in our experiment probably **disrupts the balance of immunological components that is characteristic for repair** by stimulating mononuclear cells, which leads to prolonging of inflammation." Page 4 of the English translation of Turischev (emphasis added). Accordingly, persons of ordinary skill in the art reading Turischev, as evidence by Mann, would have understood that TF5 had negative effects on tissue repair.
12. That the method of Turischev has negative effects on tissue repair such as suppuration, which is commonly known as the formation of pus. When a tissue is suppurative, it recruits and contains large numbers of neutrophils in response to an infection causing inflammation and slowing repair of the tissue. Accordingly, because Turischev discloses that topical use of TF5 increased suppuration and that suppuration existed when using TF5 intraperitoneally, persons of ordinary skill in the art would have understood that Turischev's method did not promote tissue repair.
13. Experimental evidence has shown that administration of a composition containing synthetic Tβ4 decreases neutrophil recruitment and is characterized by a lack of suppuration. Accordingly, such compositions have been found to be antimicrobial and no suppuration has been reported when administering the

composition to any subject, including rats, mice, and human subjects. In an article by Young et al. entitled "Thymosin β_4 sulfoxide is an anti-inflammatory agent generated by monocytes in the presence of glucocorticoids" published in Nature Medicine, Vol. 5(12), pages 1424-1427 (1999), the authors reported that synthetic thymosin B4 sulfoxide inhibited neutrophil chemotaxis (abstract; page 1424, right column, last paragraph). Further, in an article by Sosne et al. entitled "Thymosin- β_4 Modulates Corneal Matrix Metalloproteinase Levels and Polymorphonuclear Cell Infiltration after Alkali Injury" published in Investigative Ophthalmology & Visual Science, Vol. 46(7), pages 2388-2395 (2005), the authors demonstrated that T β_4 is a potent inhibitor of neutrophil infiltration after corneal injury. It has also been shown that T β_4 inhibits neutrophil migration in vivo (Sosne et al. "Thymosin- β_4 promotes corneal wound healing and decreases inflammation in vivo following alkali injury." *Exp Eye Res.* 2002;**74**:293–299).

14. The literature shows that "[t]he yield of thymosin β_4 from fraction 5 is about 0.45%." Low et al., Proc. Natl. Acad. Sci., 1981, page 1163, 1st paragraph of the RESULTS. Thus, the composition that Turischev administered contained only 0.45% T β_4 . The compositions administered in the experiments conducted by the inventors of U.S. patent application number 09/772,445 that showed a significant effect contained at least 10% (5 μ g/ 50 μ l) T β_4 . See Example 1 of U.S. patent application number 09/772,445.
15. Based on the Low et al. reference, I have calculated that administration of 0.8 μ g/g TF5 is equivalent to administration of 3.6 ng/g T β_4 . Thus, Turischev administered a dose of 0.79 μ g total T β_4 to the largest rats (0.8 μ g/g x 220 grams x 0.0045 = 0.79 μ g) intraperitoneally to obtain a significant wound healing effect. The experimental results detailed in U.S. Patent Application Serial Number 09/772,445 demonstrate that there was no significant effect on

tissue repair when 6 µg or less Tβ4 was administered intraperitoneally or when 2.5 µg or less Tβ4 was administered topically, (see paragraph [0100] of 09/772,445). Thus, using the same weight for the rats, I have calculated that 27.3 ng/g (6 µg/220 g = 27.3 ng/g) or less Tβ4 is not effective when administered intraperitoneally and 11.4 ng/g (2.5 µg/220 g = 11.4 ng/g) Tβ4 is not effective when administered topically. In contrast to Turischev, the inventors of U.S. Patent application 09/772,445 have disclosed a method to effectively promote tissue repair in rats by administering Tβ4 topically. Further, the inventors of U.S. Patent application 09/772,445 have disclosed a method to effectively promote tissue repair in rats by administering Tβ4 systemically by administering greater than 6 µg Tβ4.

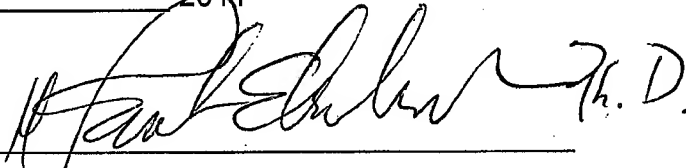
16. Turischev discloses that each TF5 dose was diluted in 0.5 mL physiological solution before being administered to the rats. Accordingly, the concentrations of Tβ4 in the compositions administered at the 0.2 µg/g, 0.8 µg/g, and 1.6 µg/g doses to the largest rat were 0.2 µg/500 µL, 0.79 µg/500 µL, and 1.58 µg/500 µL, respectively, i.e., 0.04% w/v, 0.16% w/v, 0.32% w/v, respectively.
17. It is my expert opinion that, because Turischev disclosed that TF5 was only effective at a dose of 0.8 µg/g TF5, i.e., 3.6 ng/g Tβ4, when administered intraperitoneally, it clearly did not disclose administering greater than 6 µg, i.e., 27.3 ng/g Tβ4, intraperitoneally, and taught away from modifications such as increasing or decreasing the dosage because 1.6 µg/g TF5, i.e., 7.2 ng/g Tβ4, or 0.2 µg/g TF5, i.e., 0.9 ng/g Tβ4, **were both ineffective**.
18. It is my expert opinion that persons of ordinary skill in the art reading Turischev as evidenced by Mann would not have known or been able to predict that a composition containing Tβ4 could have a positive tissue repair effect when administered topically **at any** concentration. Rather, it is clear that persons of

ordinary skill in the art reading Turischev would have learned to avoid using TF5 topically because TF5 was shown to cause "intensification and prolongation of the phase of inflammation" (page 4, lines 15-17), increased suppuration with increased TF5 dosage administered (page 4, lines 13-15), significantly slower healing and slower contraction of wounds compared to control (page 3, last two paragraphs), and "a trend toward lengthening of [the period for complete epithelialization (PCE)]" with increasing TF5 dose administered (page 5, lines 1-3).

19. All statements made herein of my own knowledge are true and all statements made on information and belief, are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 8 day of June 2011

Signed


H. Paul Ehrlich, Ph.D.

Curriculum Vitae

Name: H. Paul Ehrlich

Date of Birth: August 17, 1941

Place of Birth: Honolulu, Hawaii

Education:

- 1964 B.S. University of California at Berkeley
- 1966 M.A. San Francisco State University
- 1970 Ph.D. University of California at San Francisco

Research Fellowships and Postdoctoral Training:

1971-73 NIH-Traineeship, Departments of Biochemistry and Medicine, University of Washington, Seattle, WA

1973-75 British-American Heart Research Fellow, Strangeways Research Laboratory, Cambridge, England

Young Investigator Fellowship:

1977-81 Established Investigator of the American Heart Association

Academic and Hospital Appointments:

1994 - Present Professor of Surgery and Cellular and Molecular Physiology,
Hershey Medical Center, Penn State University College of Medicine

1984-1994 Associate Professor of Pathology - Harvard Medical School

1988-1994 Associate in Biochemistry in the Department of Surgery, and Division of Plastic Surgery, Massachusetts General Hospital

1986-1994 Associate Biochemist in the Department Pathology, Mass General Hospital

1984-1994 Clinical Research Center Affiliate, Massachusetts Institute of Technology, Cambridge, MA

1975-1984 Assistant Professor of Pathology, Harvard Medical School

1975-1986 Assistant Biochemist Department Pathology, Mass General Hospital

1975- 1993 Assistant in Biology, Shivers Burns Institute

1974-1975 Fellow Clare Hall College, Cambridge University, Cambridge, England

Other Professional Positions:

1977-1994 Director, Wound Healing Research Laboratory, Shivers Burns Institute
Massachusetts General Hospital

1994-1998 Director, Wound Healing Research Laboratory, Division of Pediatric Surgery,
Department of Surgery, Hershey Medical Center.

1998-present Director, Wound Healing Research Laboratory, Division of Plastic Surgery,
Department of Surgery, Hershey Medical Center.

Awards and Honors:

- 1954 National Outstanding Achievement Award: Ford Motor Company Industrial Arts Award in Ceramics.
- 1977-1981 Established Investigator of the American Heart Association
- 1993 Visiting Professor, Department of Pathology, University of Geneva, Switzerland
- 1995 Outstanding Service Award from the Wound Healing Society
- 1996 Visiting Professor for The Japanese Society of Plastic and Reconstructive Surgery
- 2010 Lifetime Achievement Award Wound Healing Society

Association Committee Assignments:

(Massachusetts General Hospital Committees)

- 1977-1990 Committee on Review of Research Proposals: Subcommittee of Executive Committee on Research
- 1979-1984 Committee on Animal Care: Subcommittee of Executive Committee on Research

(Hershey Medical Center Committees)

- 1994-present Surgery Department Committee of Research
- 1996-2011 Medical School Admissions Review Committee

Memberships in Professional Societies:

- | | |
|--------------|---|
| 1977- 1983 | Atherosclerosis Council of the American Heart Association |
| 1978-present | The American Society for Cell Biology |
| 1980-present | American Association for the Advancement of Science |
| 1980- 1998 | The American Burn Association |
| 1981-present | New York Academy of Sciences |
| 1989-present | Plastic Surgical Research Council |
| 1989-1992 | American Association of Pathologists |
| 1990-present | The Wound Healing Society founding member |

Major Research Interests

1. Cell biology of wound healing and scar formation.
2. Gap junctional intercellular communications and wound repair and scarring
3. Matrix and non muscle cells interactions, mechanisms and regulations of the contraction process.
4. Mediators of inflammation and dermal damage.
5. Mast Cells signaling through gap junctional intercellular communications

Editorial Advisory Board:

- 1986- Journal of Burn Care and Rehabilitation
- 1992- Wound Repair and Regeneration

Editor in Chief

- 1990-2002 Scars and Stripes (The newsletter of the Wound Healing Society)

NIH Review Committee Member

- 1989 – 1999 An Ad Hoc member of the Surgery, Anesthesia and Trauma Study Section
- 1998 Surgery, Radiology and Bioengineering Initial Review Group
- 1999 - 2002 Musculoskeletal and Dental Sciences Special Emphasis Panel
- 2001 NIH Dental & Craniofacial Research Special Emphasis Panel B
- 2003 Reparative Medicine Study Section
- 2004 Musculoskeletal Oral & Skin Sciences Special Emphasis Panel
- 2009 Musculoskeletal, Oral & Skin Sciences Integrated Review Group

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Ehrlich, H.P. (1995) Biological consideration in the repair of hernias. in "Inguinal Hernia Repair" edited by V. Schumpelick and G.E. Wantz S. Karger AG, Basel, pp 22-28.

Ehrlich, HP (1995) Is collagen remodeling associated with bladder obstruction? in "Muscle Matrix and Bladder Physiology" Plenum Publishers, New York, pp 143-149

Ehrlich HP and Gottrup F. (1998) "Experimental Models in Wound Healing" in Harding, K.G. and Leaper, D.J., eds. Wounds Biology and Management, Oxford University Press pp. 41- 51.

Iocono JA, Ehrlich, HP, Gottrup F, Leaper, D.J. (1998) "The Biology of Healing" in Harding, K.G. and Leaper, D.J., eds. Wounds Biology and Management, Oxford University Press pp. 10 - 22.

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Ehrlich HP (2001) "Possible Influence Upon Granulation Tissue Maturation by Vanadate and Implication on Grafted Cultured Skin Substitution". In R.E. Horch, A.M. Munster and B. Achauer: Cultured Human Keratinocytes and Tissue Engineered Skin Substitutes Springer-Verlag, Berlin. pp 83-90.

Ehrlich HP. (2006) The fibroblast populated collagen lattice: A model of fibroblast collagen interactions in repair. Loyola University at Chicago.

Journal Publications:

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Ehrlich, H.P., T.K. Hunt (1969) The effects of cortisone and anabolic steroids on the tensile strength of healing wounds. Ann. Surg. 170:203-206.

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Ehrlich, H.P. Tarver H (1971) Effects of beta-carotene, vitamin A, and glucocorticoids on collagen synthesis in wounds. Proc. Soc. Exp. Biol. Med. 137:936-938.

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